



## Ozone effects on wheat grain quality – A summary



Malin C. Broberg<sup>a</sup>, Zhaozhong Feng<sup>b</sup>, Yue Xin<sup>b</sup>, Håkan Pleijel<sup>a,\*</sup>

<sup>a</sup> University of Gothenburg, Department of Biological and Environmental Sciences, P.O. Box 461, SE-40530 Göteborg, Sweden

<sup>b</sup> State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, China

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### ABSTRACT

We synthesized the effects of ozone on wheat quality based on 42 experiments performed in Asia, Europe and North America. Data were analysed using meta-analysis and by deriving response functions between observed effects and daytime ozone concentration. There was a strong negative effect on 1000-grain weight and weaker but significant negative effects on starch concentration and volume weight. For protein and several nutritionally important minerals (K, Mg, Ca, P, Zn, Mn, Cu) concentration was significantly increased, but yields were significantly decreased by ozone. For other minerals (Fe, S, Na) effects were not significant or results inconclusive. The concentration and yield of potentially toxic Cd were negatively affected by ozone. Some baking properties (Zeleny value, Hagberg falling number) were positively influenced by ozone. Effects were similar in different exposure systems and for spring and winter wheat. Ozone effects on quality should be considered in future assessments of food security/safety.

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### 1. Introduction

Ground-level ozone (O<sub>3</sub>) is an important regional to semi-global air pollutant and as well an anthropogenic greenhouse gas (Simpson et al., 2014). Tropospheric O<sub>3</sub> is generated through photochemical reactions of so-called O<sub>3</sub> precursors, i.e. nitrogen oxides (NO<sub>x</sub>), volatile organic compounds (VOC) including methane (CH<sub>4</sub>), and carbon monoxide (CO), which are all emitted from anthropogenic sources. Background O<sub>3</sub> concentrations have risen from approximately 10 ppb before the industrial revolution (Volz and Kley, 1988) to daytime summer concentrations exceeding 40 ppb in many parts of the Northern Hemisphere (Vingarzan, 2004).

It is well established that current ozone concentrations are sufficiently high over large areas of the globe to cause significant adverse effects on crop production (Avnery et al., 2011; Van Dingenen et al., 2009). Wheat has been found to be a highly ozone sensitive crop (Feng and Kobayashi, 2009; Pleijel, 2011; Tai et al., 2014). There has been a strong focus on the effects of O<sub>3</sub> on

quantitative yield in crops (e.g. Feng and Kobayashi, 2009; Mills et al., 2007; Tai et al., 2014) as well as effects on photosynthesis and other physiological aspects/processes such as senescence (e.g. Feng et al., 2008; Mulholland et al., 1997; Sandelius et al., 1995) which are important for the mechanistic understanding of growth effects and for modelling. Although some studies have included quality aspects (e.g. Fuhrer et al., 1990; Pleijel, 2012; Vandermeiren et al., 1992; Zheng et al., 2013), less attention has been paid to these when it comes to research syntheses and policy related work. This paper summarises what is known about the effects of O<sub>3</sub> on wheat grain quality traits. The study is based on existing literature, but also includes some until now unpublished quality data for experiments already described in the literature, and covers experiments performed in Europe, North America and Asia.

Several quality traits exist for a crop like wheat. Some of these (primarily grain protein concentration, 1000-grain weight, volume weight, baking quality) may affect the market price of the yield. Others are nutritionally or otherwise important, but external to the market. These include e.g. the concentration of different nutrient mineral constituents and the content of the potentially toxic element Cd.

Certain aspects of wheat quality are linked to physical characteristics such as the average 1000-grain weight (technically: the

\* Corresponding author.

E-mail addresses: [hakan.pleijel@bioenv.gu.se](mailto:hakan.pleijel@bioenv.gu.se), [hakan.pleijel@me.com](mailto:hakan.pleijel@me.com) (H. Pleijel).

mass of 1000 grains) and volume weight (technically: the density or specific mass of grain). Low values of these variables indicate a large occurrence of small and malformed grains reducing the quality for use of the grain, while higher values generally are related to larger flour yield (Manley et al., 2009; Weiss and Moreno-Sotomayer, 2006). Another group of quality properties is the concentration of various nutrients including protein, macronutrients such as Mg, K, Ca, P and S as well as micronutrients like Zn, Fe, Mn and Cu. Cd is also of interest since bread is an important pathway for human intake of this potentially highly toxic, bioaccumulating element, which may have a number of important adverse health effects, for example on kidney and bone (Satarug et al., 2010). While the concentration of various grain mineral constituents is important from a qualitative perspective, also the unit area yields of these constituents are relevant for human nutrition and for biogeochemical considerations of the balance of input/output in the agroecosystems. In case the unit area yield of protein or minerals is reduced, this may significantly and negatively affect the ability of people to access sufficient protein and minerals in areas of food scarcity in addition to the loss of food energy caused by the O<sub>3</sub> impairment of grain yield. Therefore we assess both O<sub>3</sub> effects on concentrations and on the yield of these constituents.

Starch is the main source of energy in wheat grain, consisting often in the range of 60–70% of grain mass, and wheat is the second most important energy source for the human population. Thus, starch concentration was relevant to include in the study.

Finally, since wheat is used to a large extent for baking, some quality traits are related to baking quality. Here the Zeleny value, the Hagberg falling number and gluten are frequently used indicators. The Zeleny value is determined by a sedimentation test that measures the rate of sedimentation of wheat flour suspended in an acid solution. Sedimentation occurs due to swelling of glutenin, which is a major protein in wheat flour. A high Zeleny value (sedimentation volume) is associated with a high protein content and good baking quality (Eckert et al., 1993; Reeves et al., 1978). Hagberg falling number is a measure of  $\alpha$ -amylase activity in wheat grains and its resistance to enzymatic degradation. Low falling numbers represent high  $\alpha$ -amylase activity and thus more germinated grains, resulting in poor baking qualities, such as sticky dough and poorly structured loaves (Kindred et al., 2005). Additionally, a higher falling number enables longer storage time for flour and grains (Hruskova et al., 2004).

The aim of this study is to provide a comprehensive overview of all known effects of O<sub>3</sub> on wheat grain quality based on all available ecologically realistic experiments, using open-top chambers (OTC) or free air concentration enrichment with ozone (FACE) and presented as meta-analysis (to check the statistical significance of the effects) and as response regressions (to assess the magnitude of effect in relation to exposure).

## 2. Materials and methods

### 2.1. Database

Relevant papers were searched using Web of Science and selected to be included if the following criteria were fulfilled:

1. Daytime ozone concentration, [O<sub>3</sub>]<sub>day</sub>, was reported (7-h, 8-h, 12-h, 6-h or 4-h).
2. At least one of the following yield quality responses was reported: 1000-grain weight, volume weight, grain protein concentration, mineral nutrient concentration (P, K, Ca, Zn, Fe, Mn, Mg, Cu, S, Na), Cd concentration, or baking quality properties (Hagberg falling number, Zeleny value, wet and dry gluten concentrations). Grain yield data were also extracted from the

data sources to calculate yields of protein and minerals but grain yield as such is not the main focus of the present paper.

3. Ozone exposure was at least 14 days.
4. [O<sub>3</sub>]<sub>day</sub> for elevated ozone treatments was at least 30 ppb during exposure.
5. Plants were rooted in field soil, i.e. pot experiments were excluded.

The total number of wheat cultivars included in the study was 19.

After selection, a total of 36 publications were used for this meta-analysis (Appendix 1). Analyses were performed only for variables for which data existed from at least three experiments. Data used, but not available in the open literature, were mineral concentrations (ICP-Mass Spectrometry) for the Gelang et al. (2000) experiment, starch for the Pleijel et al. (1998) experiment and minerals and protein for the Chinese experiment described in Zhu et al. (2011) (minerals, protein 2007–2009 and all quality data for 2010). Data from figures were digitized using data extraction software (Get Data Graph Digitizer 2.26; <http://getdata-graph-digitizer.com/>).

### 2.2. Meta-analysis

Two parallel meta-analyses were performed for the yield quality variables, one with charcoal filtered air (CF) as control and the other with non-filtered air (NF) as control. Since FACE experiments never included a reduced O<sub>3</sub> treatment corresponding to the CF treatment used in most OTC experiments, FACE experiments could not be included in meta-analyses using CF as the control. In order to explain variation in yield quality responses, the following categories were included: (1) wheat type (spring vs. winter wheat); (2) fumigation facility (OTC vs. FACE). Parameter values were considered independent if they were made on different cultivars or O<sub>3</sub> concentrations, following the approach of previous meta-analyses (Curtis and Wang, 1998; Feng et al., 2008).

The meta-analysis was conducted using a meta-analytical software package (MetaWin2.1.3.4, Sinauer Associates, Inc. Sunderland, MA, USA) (Rosenberg et al., 2000). To estimate the treatment effect, the natural log of the response ratio ( $r = \text{variable in elevated [O}_3\text{]} / \text{variable in control}$ ) was used as the metric for analysis (Hedges et al., 1999; Rosenberg et al., 2000) and reported as the percentage changes from control as  $(r - 1) \times 100\%$  (Curtis and Wang, 1998; Feng et al., 2008). Negative percentage changes indicate a decrease in the variable in response to elevated [O<sub>3</sub>] treatment, while positive values indicate an increase.

Only a limited part of the papers reported data that would allow computation of sample variance (standard deviations or standard errors with replication). Therefore, all variables were analysed using an un-weighted approach in which the variance of the effect size was calculated using resampling techniques after 59,999 iterations (Adams et al., 1997; Feng et al., 2008; Rosenberg et al., 2000). Confidence limits around the effect size were calculated using a bootstrap method (Rosenberg et al., 2000). Estimates of the effect size were assumed to be significant if the 95% confidence intervals (CI) did not overlap zero (Curtis and Wang, 1998). The difference between categorical variables was considered significant if the 95% CIs did not overlap (Curtis and Wang, 1998; Feng et al., 2008).

### 2.3. Response functions

Response functions were derived between the relative effects of O<sub>3</sub> in the experiments vs. [O<sub>3</sub>]<sub>day</sub>. Effects were related to the effect estimated at zero [O<sub>3</sub>]<sub>day</sub> for each experiment. At zero [O<sub>3</sub>]<sub>day</sub> exposure, the biological variables were set to take the value of 1 on

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